Marker Selection in ImmPort Flow Cytometry Analysis Pipeline

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- What is marker selection?

Marker selection is a preprocessing step in the ImmPort flow cytometry analysis pipeline to allow data analysts to select a subset of markers or fluorescence channels from the originals found in FCS files for ImmPort FLOCK analysis.

- Why select markers?

There are two common scenarios in which a flow analyst may want to exclude certain markers or channels from the analysis of the FCS files.

The first scenario is when an FCS file contains unused channels.   An FCS file can record signals from fluorescence channels that are not actually stained in an FCM experiment. For example, an experimenter only stains channels FL1, FL2, and FL4 but he/she can still find channel FL3 in the resulting fcs file by default.   Another example would be fcs files which contain a complete set of scatter parameters FSC-A, FSC-H, and FSC-W while the experimenter only wanted to measure FSC-A or FSC-H.   Including unused channels in data analysis is not only unnecessary but also distorts the real data distribution, which can easily lead to sub-optimal analysis results.   The data analyzer needs to exclude unused channels before performing FLOCK analysis or cross-sample comparisons.

The second scenario is when an FCS file contains pre-gated markers.   For example, a lymphocyte gate is usually applied based on FSC and SSC.   Studies on T cells or B cells may require the data to be gated on CD3.   If the experimenter has already manually gated an FCS file before uploading it to ImmPort for FLOCK analysis, there is usually no reason to further partition the events based on these markers that have been gated.   A data analyst can decide whether he/she should include pre-gated markers into FLOCK analysis depending on whether the data should be further partitioned on these markers.

Finally, from a data analysis perspective, having a smaller number of dimensions can help avoid the occurrence of the curse of dimensionality, which can degrade the efficacy of the FLOCK algorithm as well as its performance.

- How to select markers before running FLOCK analysis?

Since ImmPort utilizes automated conversion from .fcs to .txt, the marker selection is done through allowing the data analyzer to exclude unused data columns from the .txt file(s) before performing FLOCK analysis or cross-sample comparisons.   The original .fcs files

are not affected and the ImmPort system will extract the user-specified subset of data from the .txt files for analysis and visualization.

Users can access their .fcs or converted .txt files for column selection through the Data Management and FLOCK menus. Markers and channels in an FCS-converted .txt file are displayed in columns with checkboxes.   The data analyzer can select/deselect the checkboxes to specify the columns to be included in the FLOCK analysis.   In cross-sample comparisons, data columns are automatically selected to be consistent with those in the centroid file to be applied to all files.   Selected columns are also displayed in the details of the analysis history after the analysis is done.